

Investigation of the Toxic & Teratogenic Effects of GRAS Substances to the Developing
Chicken Embryo-Report of the in-house investigations of Sodium Carbonate in the
developing chicken embryo 4/29/74

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MEMORANDUM

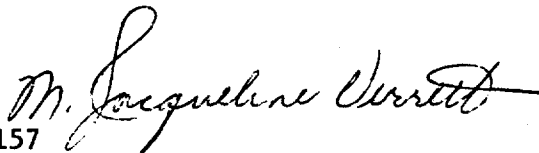
DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE
PUBLIC HEALTH SERVICE
FOOD AND DRUG ADMINISTRATION

TO : Mr. Alan Spiher
GRAS Review Branch, HFF-335

DATE: April 29, 1974

THRU : Dr. Leo Friedman, Director
Division of Toxicology, HFF-150

FROM : M. Jacqueline Verrett, Ph.D.
Reproductive Physiology Branch, HFF-157



SUBJECT: Investigation of the Toxic and Teratogenic Effects of GRAS Substances to the Developing Chicken Embryo.

Attached is the report of the in-house investigations of Sodium Carbonate in the developing chicken embryo.

Investigations of the Toxic and Teratogenic Effects of
GRAS Substances to the Developing Chicken
Embryo: Sodium Carbonate

Protocol:

Sodium Carbonate (1) was tested for toxic and teratogenic effects to the developing chicken embryo under four sets of conditions. It was administered in water as the solvent by two routes and at two stages of embryonic development; via the air cell at pre-incubation (0 hours) and at 96 hours of incubation, and via the yolk at 0 hours and 96 hours using techniques that have been described previously (2,3).

Groups of fifteen or more eggs were treated under these four conditions at several dose levels until a total of seventy-five to one hundred eggs per level was reached for all levels allowing some hatch. Groups of comparable size were treated with the solvent at corresponding volumes and untreated controls were also included in each experiment.

After treatment, all eggs were candled daily and non-viable embryos removed. Surviving embryos were allowed to hatch. Hatched chicks and non-viable embryos were examined grossly for abnormalities (internally and externally) as well as for toxic responses such as edema and hemorrhage. All abnormalities were tabulated.

Results:

The results obtained are presented in tables 1 through 4 for each of the four conditions of test.

Column 1 and 2 give the dose administered in milligrams per egg and milligrams per kilogram, respectively. (The milligrams per kilogram figure is based on an average eggs weight of fifty grams.)

Column 3 is the total number of eggs treated.

Column 4 is the percent mortality, i.e., total non-viable divided by total treated eggs.

Column 5 is the total number of abnormal birds expressed as a percentage of the total eggs treated. This includes all abnormalities observed and also toxic responses such as edema, hemorrhage, hypopigmentation of the down and other disorders such as feather abnormalities, significant growth retardation, cachexia or other nerve disorders.

Column 6 is the total number of birds having a structural abnormality of the head, viscera, limbs, or body skeleton expressed as percentage of the total eggs treated. Toxic responses and disorders such as those noted for column 5 are not included.

Column 3 through 6 have been corrected for accidental deaths if any occurred. Included in these columns are comparable data for the solvent-treated eggs and the untreated controls.

The mortality data in column 4 have been examined for a linear relationship between the probit percent mortality versus the logarithm of the dose according to the procedures of Finney (4). The results obtained are indicated at the bottom of each table.

The data of columns 4, 5 and 6 have been analyzed using the Chi Square test for significant differences from the solvent background. Each dose level is compared to the solvent value and levels that show differences at the 5% level or lower are indicated by an asterisk in the table.

Discussion:

Sodium carbonate showed moderate toxicity between 1.250 and 200 mg/kg for all four conditions of test. For air cell treatment at 96 hours the estimated LD-50 is 12.658 mg/kg (0.633 mg/egg). For the other three conditions of test the slope of the line was not significantly different ($p=0.05$) from zero and an LD-50 estimate could not be made.

There were a few serious abnormalities scattered throughout the dose levels for all four modes of treatment, however, the incidences of abnormalities were low in all instances. At only one dose level was an incidence observed that was significantly higher ($p<0.05$) than that of the solvent-treated eggs. This level was 25.0 mg/kg for air cell at 96 hours, which had five birds with serious anomalies (torticollis, 3 birds; phocomelia, 1 bird; and celosomia, 1 bird). and a few other birds with minor abnormalities of the foot and ankle.

Under the test conditions employed, sodium carbonate was neither toxic nor teratogenic between 1.250 and 200 mg/kg.

1. Sodium Carbonate, J.T. Baker Chemical Co.
2. McLaughlin, J., Jr., Marliac, J.-P., Verrett, M. Jacqueline, Mutchler, Mary K., and Fitzhugh, O.G., (1963) Toxicol. Appl. Pharmacol. 5, 760-770.
3. Verrett, M.J., Marliac, J.-P., and McLaughlin, J., Jr., (1964) JAOAC 47, 1002 - 1006.
4. Finney, D.J., (1964) Probit Analysis, 2nd Ed., Cambridge Press, Cambridge, Appendix I.

Table 1

Sodium Carbonate

Air Cell at 0 Hours

Dose		Number of Eggs	** Percent Mortality	Percent Abnormal	
mg/egg	mg/kg			Total	Structural
10.00	200.00	115	32.17*	4.34	2.60
5.00	100.00	115	16.52	3.47	0.00
2.50	50.00	113	22.12	3.53	0.88
0.50	10.00	115	14.78	4.34	1.73
0.1250	2.50	95	17.89	4.21	0.00
Water		154	13.63	0.64	0.64
Controls		333	23.12	1.20	1.20

**Slope not significantly different from zero ($p > 0.05$)

* Significantly different from solvent $p \leq 0.05$

Table 2

Sodium Carbonate

Air Cell at 96 Hours

Dose		Number of Eggs	** Percent Mortality	Percent Abnormal	
mg/egg	mg/kg			Total	Structural
5.00	100.00	105	100.00*	0.95	0.95
2.50	50.00	105	93.33*	1.90	2.85
1.250	25.00	105	89.52*	12.38*	9.52*
0.250	5.00	104	31.73	8.65	1.92
0.06250	1.25	99	29.29	8.08	5.05
Water		140	25.71	2.14	0.71
Controls		333	23.12	1.20	1.20

**I.D-50 12.658 mg/kg (0.633 mg/egg)

* Significantly different from solvent $p \leq 0.05$

Table 3

Sodium Carbonate

Yolk at 0 Hours

Dose		Number of Eggs	** Percent Mortality	Percent Abnormal	
mg/egg	mg/kg			Total	Structural
10.00	200.00	105	73.33*	0.95	0.00
5.00	100.00	105	77.14*	0.95	0.00
2.50	50.00	104	67.30*	1.92	0.00
0.50	10.00	105	72.38*	0.00	0.00
0.1250	2.50	105	59.04*	0.95	0.95
Water		115	28.69	0.86	0.86
Controls		333	23.12	1.20	1.20

**Slope not significantly different from zero ($p > 0.05$)

* Significantly different from solvent $p \leq 0.05$

Table 4

Sodium Carbonate

Yolk at 96 Hours

Dose		Number of Eggs	** Percent Mortality	Percent Abnormal	
mg/egg	mg/kg			Total	Structural
5.00	100.00	100	60.00*	6.00	6.00
2.50	50.00	100	54.00	5.00	0.00
1.250	25.00	100	43.00	5.00	1.00
0.250	5.00	100	50.00	14.00*	5.00
Water		112	40.17	0.89	0.89
Controls		333	23.12	1.20	1.20
0.06250	1.25	100	36.00	7.00*	1.00

**Slope not significantly different from zero ($p=0.05$)

* Significantly different from solvent $p \leq 0.05$